THE PRESENCE OF 5,6-DIHYDROURIDYLIC ACID IN YEAST "SOLUBLE" RIBONUCLEIC ACID

James T. Madison and Robert W. Holley

U. S. Plant, Soil and Nutrition Laboratory, Soil and Water Conservation Research Division, Agricultural Research Service, U. S. Department of Agriculture, and Department of Biochemistry, Cornell University, Ithaca, New York

Received November 2, 1964

During the analysis of pancreatic ribonuclease digests of the yeast alanine RNA a fragment was encountered that appeared to contain only guanylic acid after alkaline hydrolysis. Later an oligonucleotide was found that appeared to contain only adenylic acid and guanylic acid. Because of the known specificity of pancreatic ribonuclease it was at first supposed that a pyrimidine was involved that was unstable when subjected to alkaline hydrolysis or to the HCl used in our paper chromatography system. When the expected pyrimidine failed to appear even though the oligonucleotides were analyzed using only mild conditions of enzymic digestion, followed by paper electrophoresis or column chromatography, it seemed more likely that a "pyrimidine" nucleotide with negligible absorbancy at 260 mμ was present.

The first evidence that a nucleotide not detectable by its ultraviolet absorbance was actually present was gathered when organic phosphate was detected in column fractions that did not correspond with a peak at 260 mμ (Fig. 1, tubes 11-13). When it was learned that the material in this region gave a positive test for a dihydropyrimidine when analyzed by a method devised by Fink, et al., (1956), it was considered likely that the elusive base was a 5,6-dihydropyrimidine. For this test the material is dried on paper, sprayed with 0.5 N NaOH which opens the heterocyclic ring with the formation of a ureido group. After 30 minutes the paper is sprayed with a solution of p-dimethylaminobenzaldehyde which

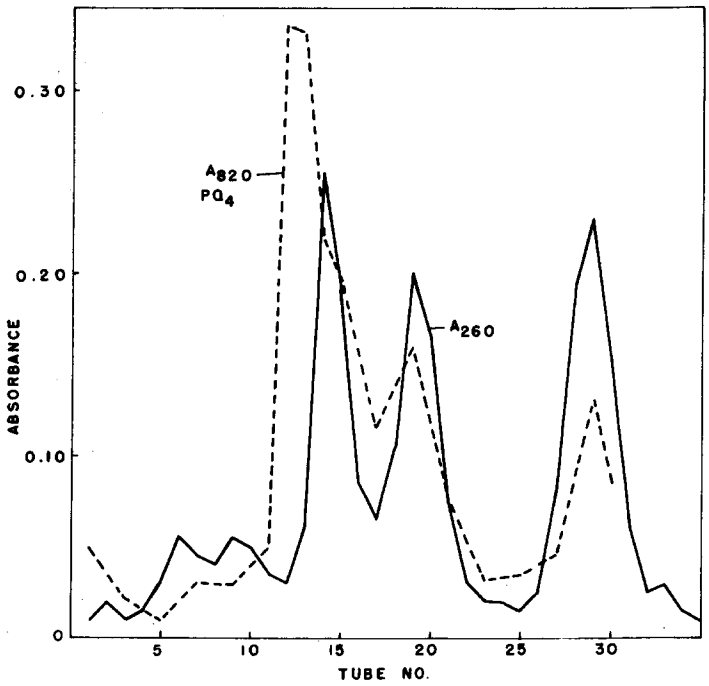

Figure 1. Chromatography on DEAE-Sephadex of the mononucleotides formed by pancreatic ribonuclease digestion of trinucleotides isolated from a ribonuclease T1 digest of alanine RNA. Elution was by a linear gradient of 0 to 0.16 M ammonium carbonate. Organic phosphate was determined by the method of Ames and Dubin (1960) and expressed as the observed A_{820}.

reacts with the ureido group to give a yellow colored product.

To obtain sufficient amounts of material for more extensive study, an attempt was made to isolate the unknown nucleotide from bulk yeast s-RNA. Fig. 2 shows that a peak that contains organic phosphate but has virtually no 260 mμ absorbance was eluted (tubes 71-75) between cytidylic acid (Cp) and pseudouridylic acid (ψp). At 230 mμ a peak in this region is visible, however. This material, dried on paper, also gave a positive test when sprayed with p-dimethylaminobenzaldehyde following NaOH. Re-chromatography on DEAE-Sephadex, as in Fig. 3, showed that most of the material emerged in a single peak (tubes 16-20). The material from this major peak, after treatment with alkaline phosphatase, was chromatographically indistinguishable from 5,6-dihydrouridine obtained from Cyclo

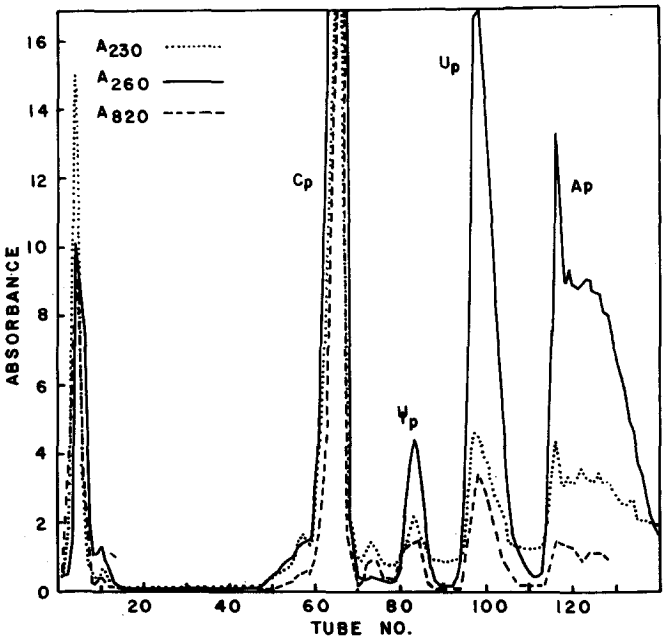

Figure 2. Chromatography of a ribonuclease T2 digest of 100 mg. of bulk yeast s-RNA on Dowex 1X8. Elution was with a gradient of ammonium formate, pH 3.5 (0, 0.1, 0.3 and 1.0 M). The peak of guanylic acid which is eluted considerably after adenylic acid is not shown.

Chemical Co., Los Angeles, Calif.[1/] in five paper chromatographic systems: (1) ethylacetate saturated with phosphate buffer (Prusoff, 1955), (2) sec-butanol saturated with water, (3) t-butanol, methylethylketone, formic acid, water, (4) t-butanol, methylethylketone, water, ammonium hydroxide and (5) phenol saturated with water (systems 2-5 as described by Cline, Fink and Fink, 1959). The Cyclo Chemical Co. dihydrouridine had Rf's in systems 2 to 5 very similar to those reported by Cline, Fink and Fink (1959) and exhibited the characteristic loss of absorption at 230 mμ in alkaline solution (Batt, et al., 1954). These results indicate that 5,6-dihydrouridylic acid was present in crude yeast s-RNA.

[1/] Trade names and company names are included for the benefit of the reader and do not infer any endorsement or preferential treatment of the product listed by the U. S. Department of Agriculture.

The ureido positive nucleotide isolated from the trinucleotides formed by pancreatic ribonuclease from alanine RNA, after alkaline phosphatase treatment, also chromatographed with dihydrouridine in these systems. This demonstrates that the unknown nucleotide from alanine RNA was also 5,6-dihydrouridylic acid. Two residues are present in the RNA.

The small first peak at tubes 9-11 (Fig. 3) gave a positive test for ureido groups after alkali treatment and contained organic phosphate, but the second small peak at tubes 13-14 was negative on both tests.

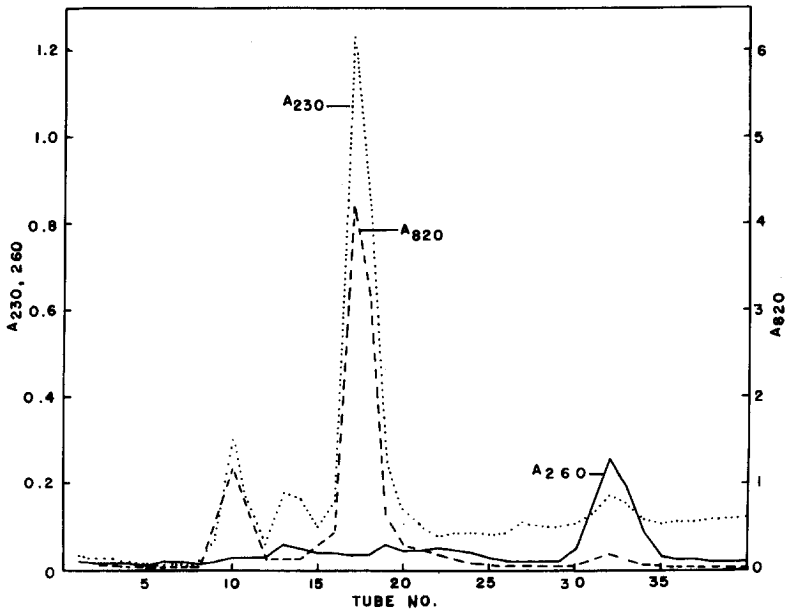

Figure 3. Rechromatography of tubes 71 to 76 (Fig. 2) on DEAE-Sephadex. Elution was with a linear gradient of 0 to 0.17 M ammonium carbonate.

The material in tubes 9-11 had an electrophoretic mobility, at pH 3.5, similar to that of uridylic acid and dihydrouridylic acid. After alkaline phosphatase treatment the nucleoside did not chromatograph with dihydrouridine. This compound has not been identified, but since in a second experiment only about one-half as much of this material was observed, as compared to the amount seen in Fig. 3, it seems possible that it was derived from dihydrouridylic acid during isolation.

This is the first report of the natural occurrence of a dihydropyrimidine in a nucleic acid.

As with all of the "minor" bases, the function of the dihydropyrimidines in s-RNA is unknown.

References

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